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The Coyote Mountains' Desert Snail (*Sonorelix harperi carrizoensis*), a Lazarus Species with the First Documentation of Live Individuals

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The Coyote Mountain desert snail (*Sonorelix harperi carrizoensis*) was described in 1937 from 30 dry shells collected the previous year. We reviewed the literature and museum records and found two additional shell collections for this subspecies from the type locality one from 1958, and one from an adjacent mountain range in 1938. There is no evidence previously of any live snails being collected from the Coyote Mountains, Imperial County, California. All shell collections of *S. harperi carrizoensis* have the same locality data as the type series, which is Painted Gorge, Coyote Mountains except for one recorded collection of shells from the Vallecito Mountains from 1938. Using geological maps and other data sources, a potential mesic habitat was identified in the Coyote Mountains. During recent field work for salamanders at this location we detected two live specimens of *S. harperi carrizoensis* approximately 2 km north of its type location. This new data confirms this subspecies is still extant and has occurred at least at two sites historically in these mountains. Despite the presence of mesic habitats (i.e., mosses, liverworts and ferns) at the type locality, we found no evidence of *S. harperi carrizoensis* or salamanders.

California has a highly diverse terrestrial snail and slug fauna of over 360 taxa, with a high number of locally endemic taxa (Roth and Sadeghian 2003). This fauna is dominated by the family Helminthoglyptidae, with several endemic desert genera including the genus *Sonorelix* (Roth and Sadeghian 2003; Goodward et al. 2017). Imperial County has the most depauperate snail fauna of any county in California with only three native snails known from the desert mountains, including two subspecies of *Sonorelix harperi* and one subspecies of *Eremarionta rowelli* (Roth and Sadeghian 2003). Two of these subspecies (*Sonorelix harperi carrizoensis* and *Eremarionta rowelli chocolata*) are endemic to Imperial County and are both poorly known in the literature or museum collections, with no evidence of recent collections for either of these endemic subspecies (Roth, pers comm.; SBNHM and SDSNH search 1 May 2020). Both subspecies were described in the 1930s from remote mountain ranges by George Willett who focused on resolving the taxonomy of these desert snails (1935, 1937), neither of these endemics have received any critical attention since these descriptions.

Similar to these snails, salamanders are also poorly known from Imperial County, with only two specimens of Southern California slender salamanders (*Batrachoseps major*) having been collected previously, each from a different mountain range [SDSNH 16316 (1933)

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and 57711 (1976)[1]]. No evidence of recent records for these salamander populations exist and with the global amphibian crisis and concern about continued species losses and impacts due to a recent extended drought, there is a need to document their occurrences (Bucciarelli et al. 2020). Springs where these species are able to persist act as biodiversity hotspots and provide important refugia for many species (Cartwright et al. 2020). The effects of climate change are poorly understood for Helminthoglyptidae (Osborne & Wright 2018), such that drought is a concern for these land snails, as it is for salamanders. Furthermore, there is increasing concern due to anthropogenic impacts in the southern California deserts, especially in sensitive mesic habitats (Lovich and Bainbridge 1999). Thus, field work is being conducted in these habitats in Imperial County to try to rediscover and assess these salamander and snail populations.

Field work included searching under surface objects during the day and searching by flashlight during the night for evidence of snails and/or salamanders in remote areas in Imperial County during the spring of 2020. This work was permitted by the Bureau of Land Management El Centro Field Office. The sites selected are based on previous descriptions of sites where salamanders were recorded. We searched topographic maps and GoogleEarth imagery to determine where damp areas, springs and seeps might occur in these desert canyons that could hold enough moisture to detect either of these taxa. We also utilized the geological tool Macrostrat (https://macrostrat.org/; Horton et al. 2017) to identify fault-lines and evidence for any pegmatite dikes where water could be forced to the surface to form a spring-type area. We utilized Calflora (https://calflora.org) to search for potential records for mesic plants such as mosses or ferns. Field work was focused on days/nights with rain in the forecast or the following week after the precipitation events to increase the likelihood of detecting the target taxa.

We identified three sites in Imperial County during March and April 2020 to search for snails and salamanders. This included one site in the In-Koh-Pah Mountains (32.629605, -116.092486), where there is a historic salamander record near Smugglers Cave, as well as two sites in the Coyote Mountains. The two sites in the Coyote Mountains are Painted Gorge (32.812875 N, -115.978887) and another canyon (32.827031, -115.992050) identified to the north where there is a clear pegmatite dike (Horton et al. 2017). The only record for the salamander from the Coyote Mountains is from 1933 and stated: "northeastern edge of the Coyote Mountains." Based on this description it seemed that the pegmatite dike that we identified using Macrostrat could be the suitable locality alluded to in the 1933 record where salamanders could have persisted. We did not find any records of mesic plants at this site from the Calfora database. Surveys have been conducted since the 1980s for salamanders at Smugglers Cave and Painted Gorge but have failed to provide any new detections. This new site had no recorded surveys until ours in 2020. The size of the usable habitat where we detected the snails at this site maps as approximately 0.2 ha.

No salamanders were detected at any of the sites surveyed. Two live snails were found at the pegmatite dike location in the Coyote Mountains and no snails were detected at the other two sites. These snails were identified to be *S. harperi carrizoensis* (Fig. 1). They were found at the top of a talus slope at the base of the dike on a north-facing slope (Fig. 2). All three sites appeared to be suitably moist, with mesic species such as mosses

[1] Fritts, T.H. 1978. The distribution, abundance, and habitat preferences of amphibians and reptiles in southwestern Imperial County, California. Interim report prepared for Bureau of Land Management, Desert Plan Staff, Riverside, California. 11 p.

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Fig. 1. Photograph of the second live individual of *S. harperi carrizoensis* recovered from the Coyote Mountains. Shell diameter is 13 mm. across. Photo credit: Samuel R. Fisher.

(e.g. *Tortula* sp.), a liverwort (*Asterella californica*), and a fern (*Myriopteris parryi*) that were clearly hydrated. Moisture was present under rocks on the surface indicating that the conditions appeared good for both snails and salamanders. No other mesic taxa of note were found underneath or outside of cover. *Sonorelix harperi carrizoensis* individuals being aestivating underneath natural cover. We also detected four shells at this new site in the Coyote Mountains, two of the shells were from where the two living snails were detected, and two were approximately 120 m upstream in the wash. We found no evidence of snails at Painted Gorge. Tissue samples were collected from the snails so that future assessment of the systematics of the population could be conducted through genetics.

The discovery of two live individuals of *S. harperi carrizoensis* confirms they still persist in the Coyote Mountains. Their detection at a new locality indicates that relic populations of the subspecies could still be surviving elsewhere in the mountains. The specimens detected were consistent with the original description of the species: <14 mm in diameter, contained only ~ 4 1/4 whorls, and shells were light-colored; compared to a maximum diameter for the type series of 14 mm with 4 2/3 whorls. This discovery indicates *S. harperi carrizoensis* is a Lazarus species. The last detection of any material from this species was in 1958. However, the fact that *S. harperi carrizoensis* has not been detected at any of the locations its shells were previously found, suggests that this species may be experiencing an ongoing range reduction, one that may be exacerbated by the current increase in drought severity (Diffenbaugh et al. 2017). Further surveys could be conducted in order to assess how widespread this species may truly be within the Coyote Mountains. Additionally, further surveys of the site where *S. harperi carrizoensis* has been detected might be conducted

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Fig. 2. The habitat where the living *Sonorelix harperi carrizoensis* were found was the line of vegetation at the bottom of the rock formation which indicated the potential for the mesic habitat for surveys. Photo credit: Robert N. Fisher.

earlier in the year to continue to try to detect salamanders in this mesic refugia. We detected snails in exceptionally wet months in 2020 where there was greater than 500% the average rainfall[2], suggesting an extreme rainfall period may be necessary to have species detections in a region that typically receives less than 100 mm annual precipitation. Finally, *S. harperi carrizoensis* current habitat should be assessed to determine if they require any management actions, as currently *S. harperi carrizoensis* is now only known from one small locality < 0.5 ha.

Adding to the usefulness of these observations, is the rarity of records for mesic bryophytes and ferns in the Coyote Mountains of Imperial County. Through Calflora we found only one record for the mountains for the fern *Myriopteris parryi* and this was from Painted Gorge from 1972 (SD83106), at the type locality for the snail. We also searched the literature for records for mosses or liverworts and found no historic records from the Coyote Mountains, possibly indicating how poorly known these mountains are for any relict mesic species (Norris and Shevock 2004; Doyle and Stotler 2006). We were able to confirm mosses, liverwort and the fern species at the site where we found the live snails, consistent with this site being a biodiversity hotspot in this xeric mountain range (Cartwright et al.

[2] California Department of Water Resources, California Data Exchange Center [online] Available at: <http://cdec.water.ca.gov/ > [Accessed 7 June 2020].

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2020). The potential use of remote sensing tools such as normalized difference vegetation index (NDVI) in these very xeric regions tied to the geological maps could prove useful in helping to remotely identifying these mesic refugia (Horton et al, 2017; Escobar-Flores et al. 2019) for further field assessment to better determine their distribution in the mountains.

Sonorelix harperi carrizoensis was first described in 1937 from dry shells, and only dry shells were collected twice after that, no living specimens have ever been recorded. We located the first living individuals of *S. harperi carrizoensis*, a cryptic species, and identified a new locality for its occurrence within the Coyote Mountain range. While no slender salamanders have been found recently, the finding individuals of this Lazarus species, *S. harperi carrizoensis*, indicates that there are still areas where desert adapted mesic species can survive in the Coyote Mountains.

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Salinity Tolerance and Osmoregulation in the Wide-spread Pacific Treefrog, *Pseudacris regilla*

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Abstract.—The effects of high saline concentrations on the plasma osmolytes of the four populations of the Pacific Treefrog, *Pseudacris regilla,* were investigated. Two of the populations, Chris Wicht and Trabuco Canyon, were in fresh water environments and two populations, Newport Back Bay and Alkaline Marsh, were in brackish water environments. The Newport Backbay treefrogs were most tolerant of increased sodium chloride solutions with 11 of 15 individuals surviving at 240 mM sodium chloride at 72 hrs. The Trabuco Canyon population was the least tolerant with 10 of 15 specimens surviving 120 mM sodium chloride at 72 hrs but none at the 180 mM concentration at 72 hrs. The Chris Wicht and Alkaline Marsh treefrogs were intermediate in their survival times with frogs surviving in 180 mM saline solutions at 72 hrs. The Newport treefrogs appeared to be the most efficient in osmoregulating by mainstreaming the plasma sodium and increasing plasma urea to compensate for the osmotic gradient produced by the increased external saline concentrations. The Trabuco Canyon and the Chris Wicht treefrogs appeared to be intermediate in their osmoregulatory abilities with the Alkaline Marsh treefrogs being least efficient and more likely an osmoconformer. The difference in osmoregulatory responses appears to be a population difference and a function of habitat. Sodium chloride was the major plasma osmolyte in the four populations of treefrogs. Urea increased in importance as the external concentration of the saline media increased, thus the role of urea was significant in maintaining the internal osmoconcentration. These simple physiological mechanisms allow this widespread treefrog to occur in so many different habitats.

The Pacific Treefrog, or Pacific Chorus Frog, *Pseudacris regilla*, formerly *Hyla regilla*, is the most wide spread Western United States' amphibian. It occurs in a variety of habitats (grasslands, woodlands, forests, farmlands, yards) from British Columbia, Canada to Baja California, Mexico (Brattstrom and Warren 1955; Dodd 2013; Jameson et al. 1966; Stebbins 2003; Stebbins and McGinnis 2012). It is occasionally found in salty or brackish salt marshes as well as at the edges of desert alkaline lakes. Since amphibian skin is so permeable to water loss, living in such salty or alkaline habitats should be perilous, unless this tree frog can tolerate high salinities and effectively osmoregulate. This hypothesis was tested using treefrogs from a variety of habitats, including a salt marsh and a desert alkaline lake.

Many workers have studied water and thermal physiology and ecology in amphibians, including water loss and terrestrialism (Brattstrom 1970; Claussen 1973; Hillman et al. 2009; MacMahon 1964; Thorson 1955), the rate of dehydration and hydration (Jameson 1966; Mclanahan and Baldwin 1969; Warburg 1965) the adaptive value of bladder

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water (Ruibal 1962) the ability to withstand desiccation (Main and Bentley 1964; Ralin and Rogers 1972; Schmid 1965; Thorson 1955) and the ability to tolerate high salinities (Albecker and McCoy 2019; Brown and Walls 2013; Gomez-Mestre and Tejedo 2003; Hopkins and Brodie 2015; Jones et al. 2015; Woolrich-Pina et al. 2015).

Pseudacris regilla is aquatic for part of its yearly activity and then has an extensive terrestrial period that includes hibernation or aestivation. Considerable geographic variation in morphology and physiology would be expected for a species that inhabits brackish marine environments, alpine meadows at 4268 m and desert ephemeral pools. To survive both terrestrial and aquatic modes of life requires extensive and varied behavioral, genetic and physiological adaptations (Jameson 1966; Jameson et al. 1966). This study was done to see what physiological adaptations the tree frogs had to these diverse habitats.

Materials and Methods

Treefrogs, *Pseudacris regilla* were collected from four study sites:

1. Trabuco Canyon, Santa Ana Mountains, Orange County, California, 33° 39' 27. 34" N, 117° 35' 06. 05" W. Flora consists of Oaks, local grasses and cattails along a streambed. Elevation: 1318 m. Freshwater.
2. Panamint Valley, Chris Wicht camp location, Panamint Mountains, San Bernardino County, California, 36° 06' 52.01" N, 117° 10' 12.46' W. Flora consists of Cottonwoods, local grasses and cattails. Elevation: 1524 m. Freshwater.
3. Alkaline Marsh Warm Sulfur Spring location, Panamint Valley, San Bernardino County, California, 36° 04' 41.99" N, 117° 13' 15.66" W. Flora consists of salt tolerant grasses. Elevation: 305 m. Brackish water.
4. Newport Backbay, Orange County, California, 33° 39' 0.00" N, 117° 53' 04.27" W. Flora consists of salt-water tolerant grasses, willows and cattails Elevation: 4 m. Salty.

Treefrogs were collected (under a California State Fish and Game Collecting Permit) by hand or net at night during the breeding season, from February through June, from 1974 to 1978. Treefrogs were kept in the lab in terraria with sand and a pan of aged tap water at 21-32 °C. The frogs were fed twice a week with *Tenebrio* Larvae and appeared healthy. All frogs were maintained under the same environmental conditions in the lab at a temperature of 13-15 °C with a 9L, 15D photo period and fed *Tenebrio* Larvae weekly. When frogs were tested, they were starved for 2 wks before the experiment. Frogs that were maintained in this manner appeared healthy and active as long as 6 mos. Only adult males and non-gravid females were used in the experiments. All Animal Care Committee rules were followed.

All saline solutions were prepared using laboratory sodium chloride (assay 99.7% pure, J. T. Baker) and distilled water. Concentrations were gravimetrically determined with 100% seawater being equivalent to 35% NaC1 or 600 mM/L about equal to 1000 m0sm/L osmotic concentration. The sample dilutions were prepared in triplicate for each concentration and were analyzed for sodium and chloride content to ensure uniformity of concentration. Each of the dilutions was contained in small jars with an 80 ml volume. The sodium chloride weight determinations were made with a Mettler P 1200 top loading balance.

Frogs were starved for 2 wks to preclude blood urea increases due to feeding. They were maintained in a moist environment during the 2 wks prior to the experiment and

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then hydrated in a container of aged tap water before being tested. The bladder water was removed by applying pressure to the pelvis area overlying the urinary bladder. After the frogs were blotted, the weight of each were determined using a Mettler Model P 1200 top loading balance with a precision of 0.005 g. The weight was then recorded, along with the time, and the frogs were completely immersed in the sodium chloride solutions which consisted of the following concentrations: 0%, 10%, 20%, 30%, 40%, 50%, 60%, 75% and 100% seawater. The seawater concentration chosen was 35 ‰ NaC1 [near the average salinity for the oceans of the world] with an osmotic concentration of approximately 1000 m Osm/liter (Gordon 1972). The seawater equivalent sodium chloride concentrations are expressed also in millimoles per liter. The frogs remained in the solutions until they either reached the critical activity point as defined by Whitford (1968), in which the frog failed to right itself within 60 secs after being placed on its back and prodded with a pencil, or until 72 hrs. At either of these points the frogs were then removed from the solutions, blotted dry, weighed and sacrificed. The plasma was then collected, the red cells separated by centrifugation and frozen at -20 °C. The plasma was later analyzed for sodium, potassium, urea and total osmolarity. The changes in the plasma potassium values of the frogs at the various concentrations were never more that 1 or 2 meq/L difference from the fresh water values.

Plasma and water sample sodium analysis were determined using an Instrumentation Laboratory 443 flame spectrophotometer, which gave a direct read-out in milliequivalents per liter. Micro dilutions were made on some samples and 20 μL of sample were required. Chloride measurements were done on the seawater dilutions, to insure uniformity, on a Corning Model 920 silver electrode chloridometer, which gave a direct read-out. Repeatability for the IL 443 includes: 0.46% RSD for sodium, 0.61% RSD for potassium, with a 90% confidence unit for 20 replicate serum determinations. The repeatability of the Corning 920 chloridometer was ± 1 meq/L for 20 determinations. Water samples were collected from the four locations and were analyzed for sodium, potassium and chloride. The sodium and potassium analysis were also determined using the IL 443 flame spectrophotometer.

Plasma urea determinations were performed on all of the collected blood samples. Two methods were employed and each gave similar results. The first method employed was the Bunstrate, from General Diagnostics, which utilized the enzyme Urease to hydrolyze urea in plasma to form ammonia and carbon dioxide. The ammonia is then reacted with the alkaline solution of sodium hypochlorite and phenol, to form indophenol. The color formed is directly proportional to the urea concentration present. The second method employed is the Urea Nitrogen Test from Dow Diagnostics. It utilized the diacetylmonoxime method, which in the presence of thiosemicarbazide, condenses urea to yield a colored chromophore which was measured photometrically using a Coleman Junior spectrophotometer.

The Osmotic concentrations were determined for all of the specimens in the salinity tolerance test. The plasma was collected in heparinized capillary tubes and sealed with Critoseal. They were then spun down using a Clay Adams microhematocrit centrifuge. The plasma was separated off and frozen. The plasma osmolarity was determined by using an Osmette A Freezing Point depression Osmometer, model number 5002. In addition, some determinations were made using a mecrolab vapor pressure osmometer, model 302. The pH of water from the Panamint alkaline was determined using a Beckman model 76 pH meter, with a sensitivity of 0.005 pH and maximum error of + 0.15. This was monitored over a two-year period from 1974-1976.

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Results

The Chris Wicht water sample was analyzed in January, April and May of the same year or of the year following. The results show that the fresh water spring is very low in sodium potassium and chloride. There was little fluctuation from January to May in the values. The Alkaline Marsh samples were collected during the same 3 mos: January, April, and May. There was more of a fluctuation in the sodium potassium and chloride values. The sodium range for the Newport Backbay was from 169 to 233 meq/L, Potassium 8.9 to 11.8 meq/L and the chloride range 157 to 168 meq/L. The Trabuco Canyon water samples ranged 10.1 to 10.4 meq/L for sodium, 0.5 to 1.1 meq/L for potassium and 6.8 to 8.4 meq/L for chloride. The seawater samples collected from Newport Beach ranged from 468 to 474 meq/L for sodium, 10.1 to 10.7 meq/L for potassium and 491 to 498 meq/L for chloride.

The data indicated that the *Pseudacris regilla* that inhabit the Newport Backbay and the Alkaline Marsh areas of the Panamint Valley were exposed to brackish water environments. The other two populations, Trabuco Canyon and Chris Wicht, inhabited fresh water environments. The results of monitoring the pH of the Alkaline Marsh indicated a range from 8.30 in November to 8.98 in July. The range was due to the rainfall in the winter months and the evaporation during the summer and early fall.

Three of the four populations of *P. regilla* were seasonal in their activity, and one, the Newport Backbay population, was active year round. The reproductive season for the Chris Wicht population of frogs was from late February through the first part of June. The Alkaline Marsh population has a breeding season that starts any time after late January and extends through June. Both populations have been observed chorusing in early September but I was unable to determine if there was reproductive activity during this time of the year. No tadpoles were observed in September. Both populations appeared inactive or dormant during the hot summer months, June through September. Both populations had a period of quiescence that extends from late October through the end of January. The Trabuco Canyon population's breeding season extended from late February through July. They were also active through the summer and fall. The Newport population were generally active throughout the year and on many occasions could be heard chorusing during the winter months.

The Newport Backbay treefrogs survived the highest sodium chloride concentration (240 mM/L, equivalent to 40% seawater) of the four populations tested. At the conclusion of the experiment (72 hrs) there were 11 of 15 specimens alive and active. The Trabuco frogs were the least tolerant with 8 of 12 frogs surviving in 120 mM/L sodium chloride solution (equivalent to 20% seawater). At 180 and 240 mM/L NaC1 solutions there were no survivors at 72 hrs. The Chris Wicht frogs were tolerant of 180 mM/L NaC1 solutions (equivalent to 30% seawater) with 8 of 15 specimens surviving at 72 hrs. There were no survivors at 240 mM/L NaC1. The Alkaline Marsh frogs were tolerant of 180 mM/L NaC1 (equivalent to 30% seawater) with 7 of the 10 frogs surviving at 72 hrs. The survival times of the four populations of *P. regilla* differed at each concentration of sodium chloride. At 240 mM/L NaC1, frogs from the Newport Backbay were alive at 72 hrs; however, there were no survivors at that concentration from the other three populations. The Chris Wicht and Alkaline Marsh *P. regilla* had members surviving in 180 mM/L NaC1. This was intermediate to the Newport and Trabuco Canyon frogs in tolerance to increased sodium chloride concentrations.

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The plasma urea levels were determined on the blood samples from each population of Hylas at each concentration. The data indicate that as the NaC1 concentrations increase, the plasma urea values also increased. The amounts of plasma urea varied with each population and they all showed a continuous rise to 240 mM/L NaC1, at that point the Trabuco population had the highest mean value, which was above 60 mM/L urea. The plasma urea levels for the Alkaline Marsh population, the Chris Wicht population, and the Newport Backbay population showed a peak at 240 mM/L NaC1. Between 240 and 360 mM/L NaC1 the four populations showed decrease in mean values, but at these concentrations, the mean survival rates drop rapidly. The peak urea values appear at NaC1 concentrations tolerated by each population of *P. regilla* and at a point where there were significant decreases in the mean survival times of each.

The effects of increased sodium chloride concentrations on the plasma sodium levels were determined for populations of *P. regilla*. The Newport Backbay and Chris Wicht populations have mean sodium values between 170 and 180 meq/L. The Trabuco population had a mean value of 135 meq/L and the Alkaline Marsh population had a mean value of 126 meq/L. The Alkaline Marsh population showed a continuous rise in plasma sodium values to 240 mM/L NaC1. At the 240 mM/L NaC1 concentration the mean plasma sodium value was 320 meq/L which was more than double the mean value in fresh water. The mean survival times for the Alkaline Marsh population dropped rapidly at this level. The Chris Wicht population showed a rise in the mean sodium value to 206 meq/L. At 120 mM/L NaC1 concentration, the mean sodium value decreased to 121 meq/L. At 180 mM/L NaC1 concentration, the mean sodium values increased to 142 meq/L. The mean survival time decreased rapidly from 180 mM/L NaC1. The mean plasma sodium concentration of the Trabuco Canyon population droped from a mean value of 135 meq/L in fresh water, to a mean concentration of 87 meq/L at 60 mM/L NaC1. At 120 mM/L NaC1 the mean plasma sodium value was 136 meq/L and continued to climb to 360 mM/L NaC1. At 120 and 180 mM/L NaC1 the Newport population maintained a mean value of 153 meq/L. It rose to 196 meq.L at 300 mM/L NaC1 and dropped to a mean value of 183 meq/L at 360 mM/L NaC1. The mean survival times do not start to fall off until 240 mM/L NaC1. At 240 mM/L NaC1 there were 11 of 15 animals alive after 72 hrs of submersion. It was interesting that the Newport and Trabuco populations showed a temporary decrease in mean sodium values at 60 mM/L NaC1 and then both increased again at 120 mM/L NaC1. The Chris Wicht population at 120 mM/L NaC1 showed a decrease. The Alkaline Marsh population showed no decrease, only a steady increase in mean sodium values as the NaC1 concentrations increase. Similar patterns were evident in plasma urea values as the NaC1 concentrations increase.

The effects of increased sodium chloride concentrations on plasma osmolarity were determined for each population of *Hyla regilla.* The Newport population had a mean plasma osmolarity of 346 mOsm/L in fresh water. It showed a decrease to 288 mOsm/L at 60 mM/L NaC1. At 120 mM/L NaC1 there was an increase again which continued to 600 mM/L NaC1. The Trabuco population had a mean plasma osmolarity of 274 mOsm/L in freshwater but decreased to a mean value of 177 mOsm/L in 60 mM/L NaC1. At 120 mM/L NaC1 the osmolarity again increased and was continuous to 360 mM/L NaC1. The Chris Wicht population had a mean plasma osmolarity of 354 mOsm/L in freshwater. The osmolarity increased at 60 mM/L NaC1 to 407 mOsm/L then decreased to 254 mOsm/L at 180 mM/L NaC1. At 180 mM/L NaC1 the osmolarity increased and continued to increase to 450 mM/L NaC1. The osmolarity of the Alkaline Marsh population had a mean value of 260 mOsm/L in fresh water and increased continuously to 300 mM/L

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NaC1. The comparative patterns were similar for the Newport and Trabuco populations with an initial decrease in plasma osmolarity from fresh water to 120 mM/L NaC1. In the Chris Wicht population the decrease was from 60 to 12 mM/L NaC1 and the increase from 120 to 180 mM/L NaC1. The Alkaline Marsh population showed no decrease, only a continuous increase.

Discussion

Anurans have been shown to have a limited tolerance to salt or brackish water (Ruibal 1962; Gomes-Mestre and Tejedo 2003; Brown and Wall 2013; Hopkins and Brodie 2015; Jones, et al. 2015; Woolrich-Piña et al. 2015). Only a few euryhaline and no marine species are recognized (Gordon, 1972). The crab-eating frog *Rana cancrivora* and the European green toad, *Bufo viridis* are considered exceptional in their ability to tolerate exposure to hyperosmotic media, compared to other species studied that cannot withstand even moderately brackish water, beyond 15 0/00 salinity or 30 to 40% seawater or higher (Gordon 1972). Generally, most amphibians are unable to adapt to salinities higher than 0.7 to 0.9% NaC1 (Bentley 1971; Katz 1975; Hillman et al. 2009).

Some species of anurans are able to tolerate higher concentrations of sodium chloride if they are gradually acclimated compared to being subjected to immediate hypersmotic stress. Katz (1975) found that *Rana ridibunda* acclimated to increased salinities could tolerate up to 300 mOsm/L (about 25% seawater) and the plasma remained hyperosmotic to the external media. Romspert (1976) found that *Xenopus laevis* could be adapted to saline solutions above 460 mOsm/L but exhibited a 50% mortality rate at concentrations of 460 to 515 mOsm/L (40 to 45% seawater). Munsey (1972) found that acclimated *Xenopus laevis* could withstand up to 240 mM/L sodium chloride (40% seawater) for a period of 82 hrs. *Xenopus laevis* increases the plasma urea nitrogen in response to the hyperosmotic stress; however, Katz (1975) found that *Rana ridibunda* is unable to accumulate urea, while *Xenopus laevis* can (Munsey1972; Romspert 1976).

The *P. regilla* used in this study were not acclimated to the hyperosmotic saline solutions. They were subjected to the hyperosmotic saline solutions directly by forced immersion. The Chris Wicht and Trabuco Canyon populations of treefrogs have to cope with hyperosmotic environments and are faced with possible loss of body water in their aquatic environments as well as their terrestrial environments. The Alkaline Marsh was the equivalent of about 10% seawater (60 mM/L NaC1). Frogs from each of the four localities were immersed in saline solution of sodium chloride and were subjected to the same hyperosmotic stress. They were placed in containers in which only their heads could rise out of the solutions. After 72-hrs of submersion, 11 of 15 Newport frogs were alive and active in 40% seawater (240 mM/L NaC1). Trabuco frogs had 8 of 12 surviving at 72 hrs in 20% seawater (120 mM/L NaC1). Chris Wicht had 8 of 15 frogs alive at 72 hrs in 30% seawater (180 mM/L NaC1) and the Alkaline Marsh had 7 of 10 frogs alive at 72 hrs at the same concentration.

The Newport Backbay brackish water frogs, had a greater tolerance of osmotic stress that the other three populations at 240 mM/L NaC1. The Newport frogs had a mean survival time of 19.3 hrs compared to 12.8 hrs in the Alkaline Marsh frogs, 7.33 hrs in the Chris Wicht frogs, and 3.40 hrs in the Trabuco frogs. The data from the analysis of plasma samples for urea indicate that all four populations have the ability to accumulate urea in the plasma in response to hyperosmotic stress. The increases in plasma urea are in direct response to the increase in sodium chloride solution up to 300 mM/L NaC1 for all four populations. The patterns are similar for the four populations though the plasma levels

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differ. It is evident that as the outside concentration increases, the plasma urea increases. All four populations of frogs have the ability to accumulate urea, though the survival time for the four populations in response to increased hyperosmotic stress is different. It has been established that urea develops when frogs are placed in high salinities in many frog species. The tolerance of the aquatic anuran *Xenopus laevis* to hyperosmotic solutions has been shown to be a result of an increase in urea and blood electrolytes (Belinsky et al. 1961; Jansen and Cohen 1968; McBean and Goldstein 1967).

The ability to produce and to store high concentrations of urea is considered advantageous to amphibians in osmotically stressful environments (McClanahan 1975). Increasing the osmolarity to reduce the osmotic gradient would aid in reducing the water loss. The data suggests that urea in the plasma is increased in response to hyperosmatic stress and that it is one of the main factors responsible for the increase in osmolality in *P. regilla*. The mechanism involved in increasing the plasma urea in *P. regilla* is not known. It may be due to renal retention of urea, renal shut-down due to dehydrating stress, increased synthesis through heightened activity of the enzymes involved in the ornithine-urea cycle, or a combination of the above factors. The response appears to be physiological in nature and directly related to hyperosotic stress.

The plasma sodium values of frogs in fresh water were higher than that of the environment. At 30% seawater (180 mM/L NaC1) there was a shift in the plasma sodium values compared to the environment. In all but the Alkaline Marsh population, the sodium values become less that the environmental sodium values. In the alkaline marsh population, the Chris Wicht frogs have the lowest mean plasma sodium values of the four populations. They appear as the most euryhaline of the four populations of frogs. The Trabuco frogs appear to be the most stenohaline of the four populations. The Chris Wicht frogs were intermediate in sensitivity to increases in sodium chloride concentrations in terms of survival times (Hillman et al. 2009). When subjected to hyperosmotic stress, three of the populations of *P. regillis* (Newport, Trabuco, and Chris Wicht) appear to show some attempt to osmoregulate. The Newport brackish water population appears to be the most efficient at doing so based on its ability to accumulate urea to increase the osmoconcentration and to maintain or hyporegulate its plasma sodium. The Chris Wicht and Trabuco populations appear to be partial osmoregulators and the Alkaline Marsh population appears to be an osmoconformer. The Trabuco population appeared to be the most sensitive to the stress of hypersalinity and the Newport population the least sensitive to the stress of hypersalinity. Thus in comparing the two brackish water populations (Newport and Alkaline Marsh), there appears to be two contrasting ways to cope with hyperosmotic stress. The Newport population appears to do so by osmoregulating and the Alkaline Marsh does so by osmoconforming. In the Newport population regulating the plasma sodium concentration appears to be an important mechanism in osmoregulation. In Alkaline Marsh population this does not appear to be as important. The Chris Wicht and Trabuco populations appear to be doing some osmoregulating of plasma sodium, up to 150 mM NaC1 based on the data in this experiment. They appear intermediate and the osmoregulating Newport Back Bay population. These simple physiological mechanisms allow this widespread tree frog to occur in so many different habitats (Jameson 1970; Hillman et al. 2009).

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